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Studies on *Cryptogramma crispa* Spore Germination

EMILIA PANGUA, LORENA GARCÍA-ÁLVAREZ, and SANTIAGO PAJARÓN
Departamento de Biología Vegetal I, Facultad de Biología, Universidad Complutense, E-28040 Madrid, Spain

ABSTRACT.—In order to study the germination capacity of *Cryptogramma crispa*, spores were cultured on sterilized Petri dishes with nutritive medium solidified with agar. Germination was checked at 10, 15, 20 and 25°C, and, as in most of homosporous ferns, the germination optimum was at temperatures above 20°C. Two light intensities were used, 10 and 40 $\mu Em^{-2}s^{-1}$, to reproduce the possibilities of the spores falling on open sites or in rock cracks or hollows. A lower light intensity accelerates germination. After sowing, some plates were kept at chilling and other at freezing temperatures to check the effect of low storage temperatures on the germination capacity of the spores. After these processes, the spores are able to germinate and reach similar germination rates, although the frozen spores delay the beginning of germination and show a decreased germination rate. The results of these experiments point toward the possibility that the spores of *C. crispa* are dispersed at the end of the growing season and go through a dormancy until next spring.

In the Iberian Peninsula (Spain and Portugal), *Cryptogramma crispa* R. Br. grows on ecologically very particular and well defined habitats. It occupies especially siliceous stone fields, such as granite, gneiss, sandstones, quartzites, or slates in high mountain zones, usually above the timber-line. In the Iberian Peninsula, its optimum is about 2000 m. The plants grow preferentially in rock fissures or cracks and in hollows between rock blocks. In these habitats, which are mostly over 2000 m elevation, the growing season may be very short, scarcely two months in extreme conditions, and usually no more than four months (Rivas-Martínez, 1987).

The considerations of the distribution of pteridophytes suggest the need for more detailed investigations on the life-cycles of species to determine the importance of specific variations in the life-cycle in limiting the distributions of plants. Variations in the distributions of species might be accounted for by random processes, such as dispersal, or in a more deterministic manner by subtle and specific variations in life cycle characteristics (Woodward, 1987). In ferns, it is important to study the factors that can affect the development of the gametophyte that would lead to the establishment of the sporophytic generation.

Probably due to the short period to complete their development, *C. crispa* sporophytes reach the end of the summer with practically all the spores still retained (Peck et al., 1990; pers. obs.), which are released at about the same time that the leaves shed. Thus, the spores are released at the end of the growing season.

It is difficult to reproduce wild conditions in short-term laboratory experiments. But some climate changes, especially temperature ones, that may influence spore germination can be tested in the laboratory, provided that in nature conditions may be operating over a longer period. Thus, a few experiments

TABLE 1. Localities of and acronyms for populations of *Cryptogramma crispa* used in the present study. Except for population SCO, the localities are all located in Spain.

Acronym	Locality
HU	Prov. Huesca, Torla, Barranco de la Pazosa, slates, 2000 m, *Herrero* s.n. on 2 Oct 1995.
LNE	Prov. Soria, Sierra de Urbión, Laguna Negra, 1900 m, *Pajarón & Pangua* s.n. on 26 Sep 1996.
MON	Prov. Zaragoza, Sierra del Moncayo, near Ermita de la Virgen, 1620 m, *Prada & Pangua* s.n. on 4 Oct 1996.
PEÑ	Prov. Madrid, Sierra de Guadarrama, Peñalara, granite stone field, 2100 m, *Pajarón & Pangua* s.n. on 10 Oct. 1996.
SCO	SCOTLAND, Central Region, near Aberfoyle, slate stone field, 550 m, *Jermy, Pajarón, Pangua & Lindsay* s.n. on 28 Aug 1995.
STI	Prov. Soria, Sierra de Freguela, near Puerto de Santa Inés, siliceous stone field, 1799 m, *Pajarón & Pangua* s.n. on 25 Sep 1995.

were designed to check how temperature and light intensity affect spore germination, how low storage temperatures can change spore viability and whether spores are able to undergo a dormancy until spring, and if more or less sharp temperature changes can affect spore germination rate. These experiments are described below.

MATERIALS AND METHODS

We have used spores from six populations at different localities scattered in the distribution area of this species in the Iberian Peninsula. We also included a population from Scotland, which originated from a similar habitat but at lower elevation. The localities of the populations studied are listed in Table 1. Climatic data at the nearest meteorological stations were obtained from Elias Castillo & Ruiz Beltrán (1977). The climatic conditions in Scotland were similar to those at the Iberian populations because of increased latitude. All spores were stored at a room temperature of 20°C.

To study germination, the spores obtained from several plants in each population were sown on sterilized 6 cm diameter Petri dishes with nutritive medium solidified with agar (Dyer, 1979). For each of the following culture conditions and each locality two dishes were sown. In all cases, the germination rates were calculated by counting 50 spores from each plate and expressed as the mean value of the two dishes. Germination was scored as spores in which the first rhizoid had emerged. In the four experiments we used white light and a 12 hour light/dark photoperiod. Temperature and light intensity conditions varied in the four experiments as described below. In experiment 1, spores from all the populations were studied. In experiments 2–4, we used only spores from the SCO, STI, and HU populations.

EXPERIMENT 1.—Plates were cultivated at $30\mu Em^{-2}s^{-1}$ and at 10, 15, 20, and 25°C. The lowest temperature was chosen because spring months are still cold, and mean temperatures of about 10°C in May are common at these localities (Elias Castillo and Ruiz Beltrán, 1977). This experiment was developed to

check the effects of temperature on the germination of spores, and to check if the spores could initiate germination at low temperatures, similar to the usual mean temperatures ocurring at the begining of spring.

EXPERIMENT 2.—The temperature was maintained at 12°C for 31 days and then raised to 18°C, with samples cultivated at $10\mu Em^{-2}s^{-1}$ and at $40\mu Em^{-2}s^{-1}$. Sharp changes in temperature are not uncommon in montane climates. We chose this jump from 12° to 18°C because these are the usual mean temperatures in June and July respectively at these localities (Elias Castillo and Ruiz Beltrán, 1977). We were attempting to check if this kind of change could accelerate the germination process. The different light intensities were used to check if there is an influence of this parameter on germination of the spores. Considering the habitat of these plants, it is possible for the spores to fall either on the rock surface or the soil, where they are exposed to direct sun-light, or in rock fissures and under rock blocks, where illumination is lower.

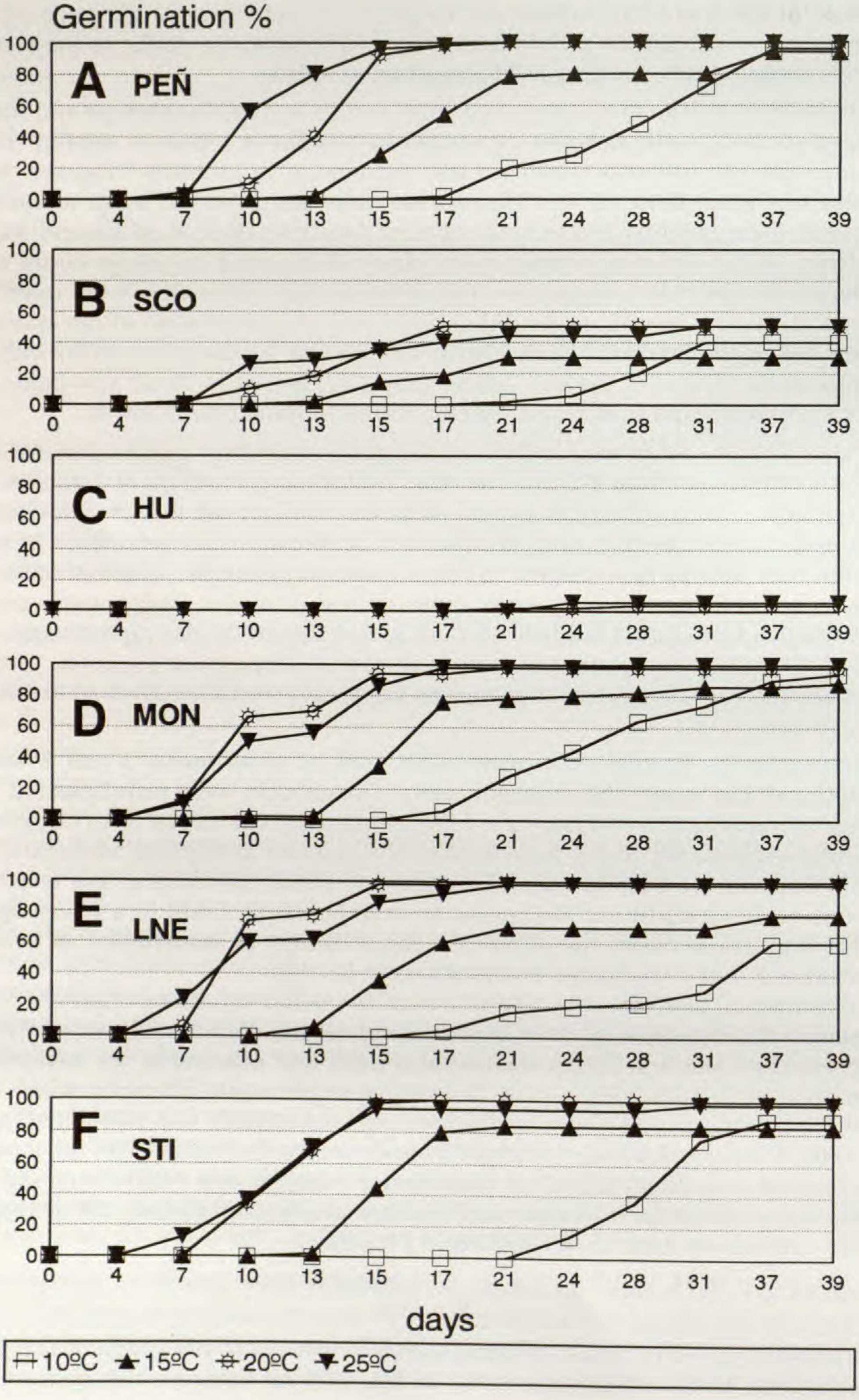
EXPERIMENT 3.—After sowing, half of the plates were kept in darkness for 15 days in a refrigerator at 4°C and the other half received 15 days of darkness in a freezer at −18°C. After this period, all plates were moved to a growth-chamber and cultured at 21°C and 30 $\mu Em^{-2}s^{-1}$. If spores do not germinate in autumn, they should be subjected to low winter temperatures, although most of them should be protected by snow cover at these altitudes. Under snow cover, the temperatures rarely fall below freezing, and this cover also protects against wind action and desiccation as there is almost no evaporation (Geissler, 1982). In case no snow cover protection occurs, the spores would be exposed to freezing temperatures.

EXPERIMENT 4.—In this experiment conditions of experiments 2 and 3 were combined, but at low light intensity only. The cultures were maintained at 10 $\mu Em^{-2}s^{-1}$ and 12°C for 14 days, then the temperature was raised to 18°C. Before the experiment, the plates were kept at chilling and at freezing temperatures as in Experiment 3 for 15 days. We wanted to check the effect of low temperatures on the viability of the spores as in Experiment 3, but at a lower light intensity, and to check the effect of a sharp change in temperature as in experiment 2, but with spores previously kept in cold.

A statistical analysis was carried out to test if the variations observed represent real differences between treatments or merely chance differences. First, we searched the best fitting regression model, and afterwards we compared the regression lines within, ever, and among experiments when necessary. To test the goodness of fit of the model, the r-squared statistic was calculated, and also the F-ratio and p-value obtained from the analysis of variance of the model were used. A further analysis of variance for variables was carried out, and F-ratio and p-values for intercepts and slopes were obtained as well. All analyses were carried out with STATGRAPHICS Plus 3.0.

RESULTS

EXPERIMENT 1.—The results of germination experiments at a range of temperatures from 10 to 25°C are presented in Fig. 1. In all samples, the spores cul-


Germination %
A PEÑ
B SCO
C HU
D MON
E LNE
F STI
100
80
60
40
20
0
0 4 7 10 13 15 17 21 24 28 31 37 39
days
10ºC 15ºC 20ºC 25ºC

tivated at 20° and 25°C began germination four days after sowing, and after two weeks practically all viable spores had germinated. At 15°C, germination was delayed about a week, and the delay was greater in 10°C cultures, in which germination started 15–20 days after sowing. Population HU showed a different behavior (Fig. 1c), as only spores cultivated at 20° and 25°C germinated and the germination rate was less than 10%.

Higher germination rates varying from 84–100% were reached in cultures at 20° and 25°C, except in SCO, which only reached 50% (Fig. 1b). In cultures at 10° and 15°C, PEÑ and MON (Fig. 1a, d) had relatively high germination rates at the end of the experiment, similar to the rates obtained at higher temperatures. Nevertheless, in SCO and STI (Fig. 1b, f) a small decrease of spore viability was observed at 10° and 15°C and a greater decrease was seen in LNE (Fig. 1e).

Statistical analysis (Table 2) shows no significant differences among the slopes, but highly significant ($p<0.01$) differences among intercepts, comparing the results of each population at each culture temperature.

EXPERIMENT 2—Germination rates from SCO, STI, and HU kept at the same temperature but at two different light intensities were similar in both cases. The germination rates varied from 85–95%, except in HU. However, at a higher light intensity (Fig. 2a), germination started 22 days after sowing, whereas at 10 $\mu Em^{-2}s^{-1}$, it began four days earlier (Fig. 2b). After day 31, at which temperature increased from 12 to 18°C, spore germination rates increased abruptly in all samples.

No significant differences are shown in the statistical analysis between both light intensities, except for population STI (Table 2). However, there are highly significant differences when compared with the results of experiment 1.

EXPERIMENT 3.—Half of the replicates were kept in darkness at 4°C for 15 days (Fig. 3a) and the other half were kept in darkness at −18°C for 15 days (Fig. 3b), before transfer to a growth chamber at 21°C and 40 $\mu Em^{-2}s^{-1}$. The spores kept at 4°C began germinating 5 days after transfer to the growth chamber, and in a few days reached their highest germination rates. The spores kept at −18°C began germinating 12 days after transfer to the growth chamber. The highest germination rates were reached a few days after transfer to the growth chamber. In all samples, the germination rates were clearly lower in samples exposed to freezing temperatures, especially in SCO, in which germination decreased from 80% to 10%.

In both treatments, STI had the highest germination rates, followed by SCO, with the lowest germination rates in HU. Comparing these results with the ones obtained in experiment 1, it is apparent that SCO lost viability when kept at 4°C, and much more so if spores were kept at −18°C, relative to spores

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FIG. 1. Germination rates of all six populations at 10, 15, 20, and 25°C (experiment 1), during 39 days.

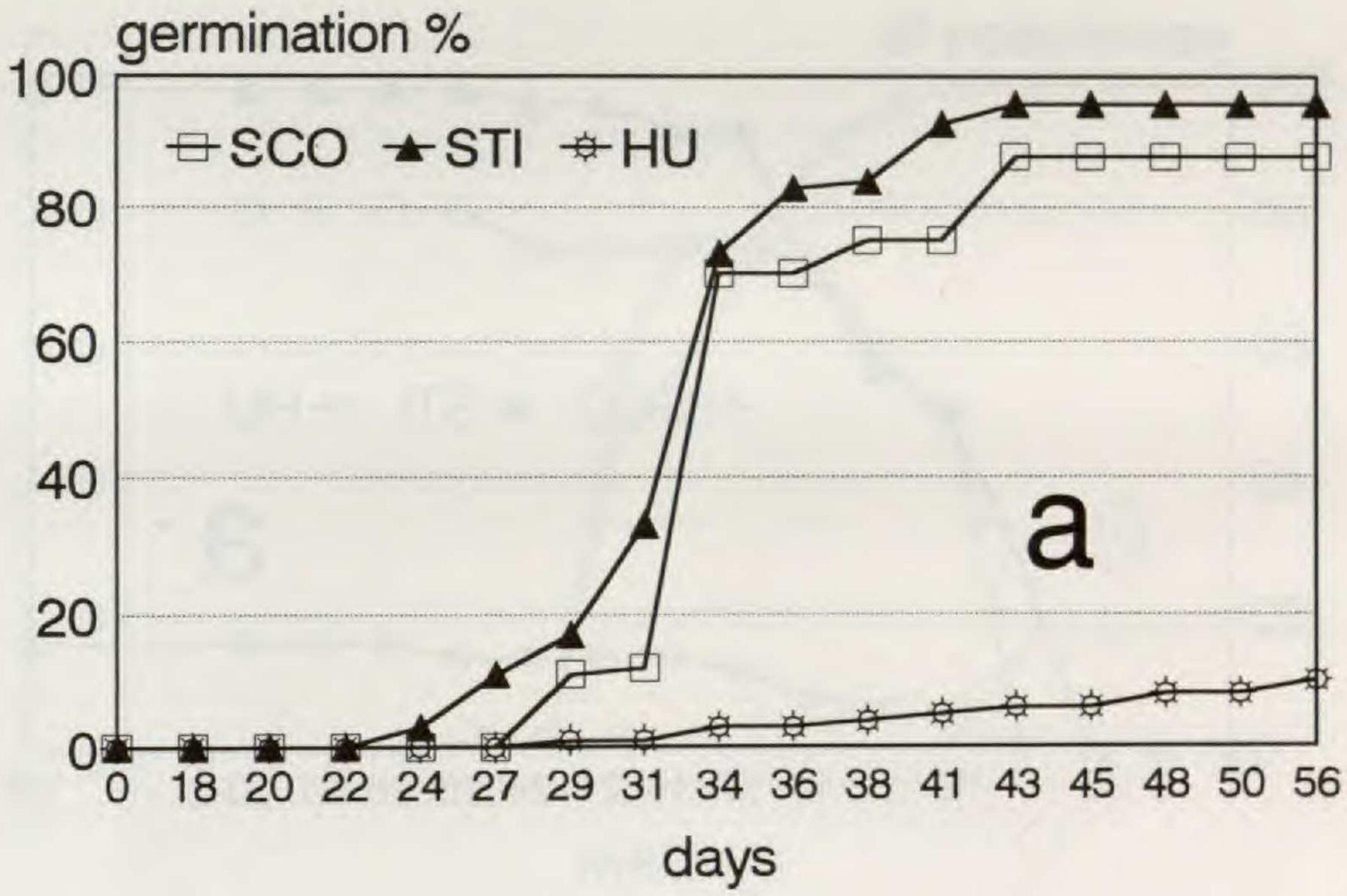
TABLE 2. Results of the statistical analyses of each experiment and of comparison between experiments. Columns 2–4: r^2 statistic indicating the percentage of variability explained by the model as fitted; F-ratio and p-value are results of the analysis of variance of the model. Columns 5–8: F-ratio and p-value for intercepts and slopes are results of the further analysis of variance for variables.

				Intercepts		Slopes	
Population	r^2	F	p	F	p	F	p
Experiment 1							
PEÑ	78,09	38,30	0,0000	11,961	0,0000	0,35	0,7901
SCO	84,27	30,61	0,0000	21,66	0,0000	1,51	0,2259
MON	83,80	29,56	0,0000	16,48	0,0000	0,11	0,9543
LNE	87,99	41,89	0,0000	44,68	0,0000	2,31	0,0911
HU	84,21	30,47	0,0000	23,75	0,0000	24,70	0,0000
STI	80,30	23,29	0,0000	15,62	0,0000	0,40	0,7514
Experiment 2							
SCO	85,40	54,61	0,0000	0,37	0,5470	1,64	0,2111
STI	91,26	97,42	0,0000	7,86	0,0091	4,26	0,0483
HU	93,47	133,63	0,0000	0,17	0,6842	1,07	0,3103
Experiment 2 vs Experiment 1							
SCO	86,66	44,56	0,0000	7,52	0,0003	8,22	0,0002
STI	84,88	38,49	0,0000	27,84	0,0000	11,38	0,0000
HU	94,32	114,02	0,0000	10,58	0,0000	51,08	0,0000
Experiment 3							
SCO	96,68	252,76	0,0000	535,99	0,0000	90,29	0,0000
STI	87,26	59,37	0,0000	23,38	0,0000	0,85	0,3639
HU	95,63	189,53	0,0000	323,91	0,0000	104,35	0,0000
Experiment 4							
SCO	85,17	49,78	0,0000	67,09	0,0000	33,46	0,0000
STI	85,24	50,07	0,0000	23,11	0,0001	4,55	0,0426
HU	83,91	42,95	0,0000	36,28	0,0000	37,52	0,0000
Experiment 3 vs Experiment 4 (−18°C)							
SCO	78,88	32,38	0,0000	0,62	0,4372	0.92	0,3473
STI	85,00	49,10	0,0000	15,46	0,0006	0,72	0,4030
HU	41,17	6,06	0,0028	0,85	0,3646	0,92	0,3466
Experiment 3 vs Experiment 4 (4°C)							
SCO	82,28	40,23	0,0000	8,31	0,0078	1,17	0,2892
STI	83,14	42,74	0,0000	6,25	0,0190	0,18	0,6750
HU	85,12	49,58	0,0000	26,29	0,0000	0,19	0,6669

maintained at room temperature. The same phenomenon occurred in STI, but at a lower proportion.

These results show statistically significant differences among intercepts and among slopes, except for population STI, in which differences between the slopes of both treatments were not significant (Table 2).

EXPERIMENT 4.—As in experiment 3, after sowing the plates were maintained at 4° and −18°C. Afterward, they were cultivated for 14 days at 12°C followed by cultivation at 18°C, always at 10 $\mu Em^{-2}s^{-1}$. Results are shown in Fig. 4.



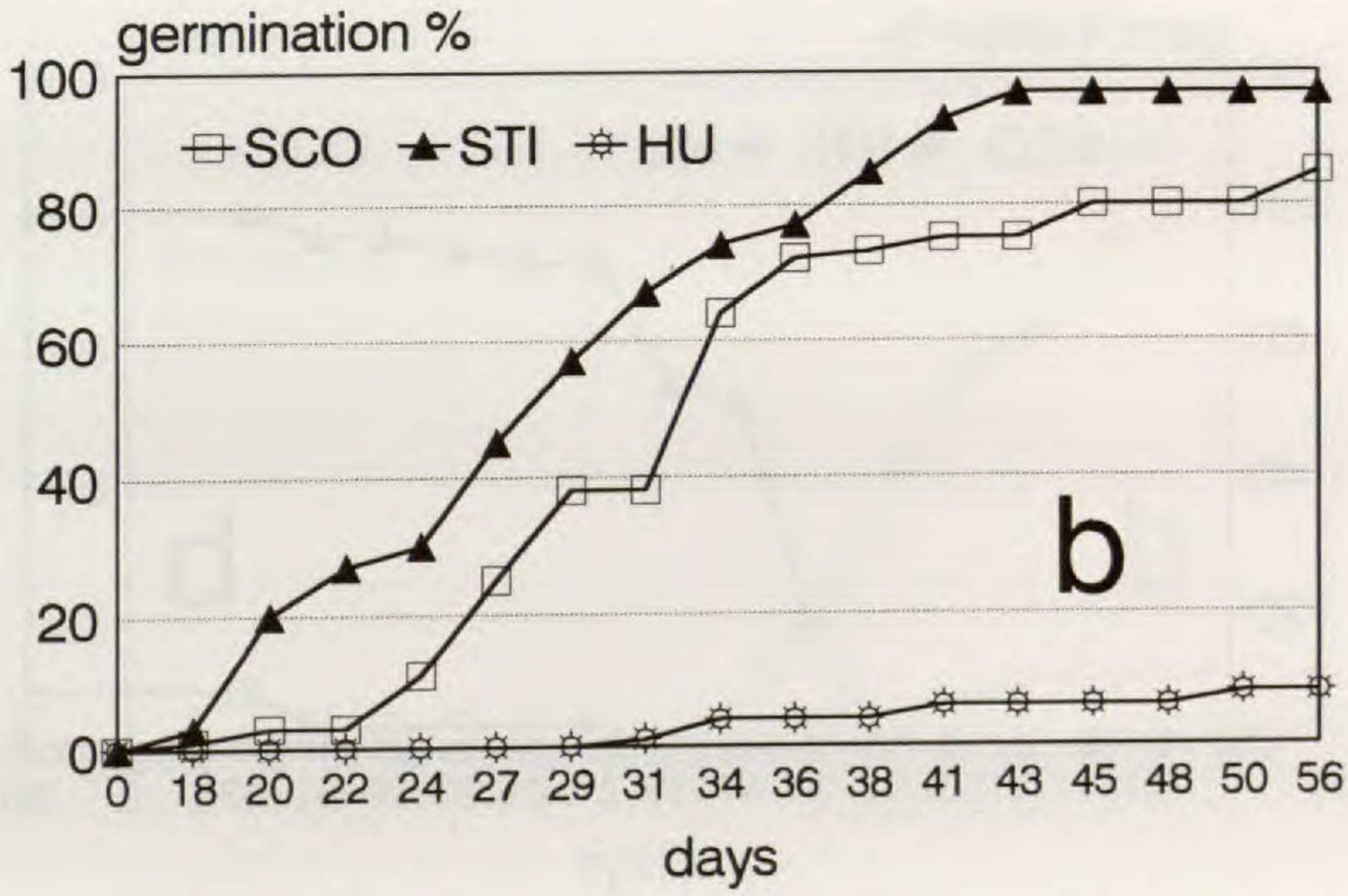
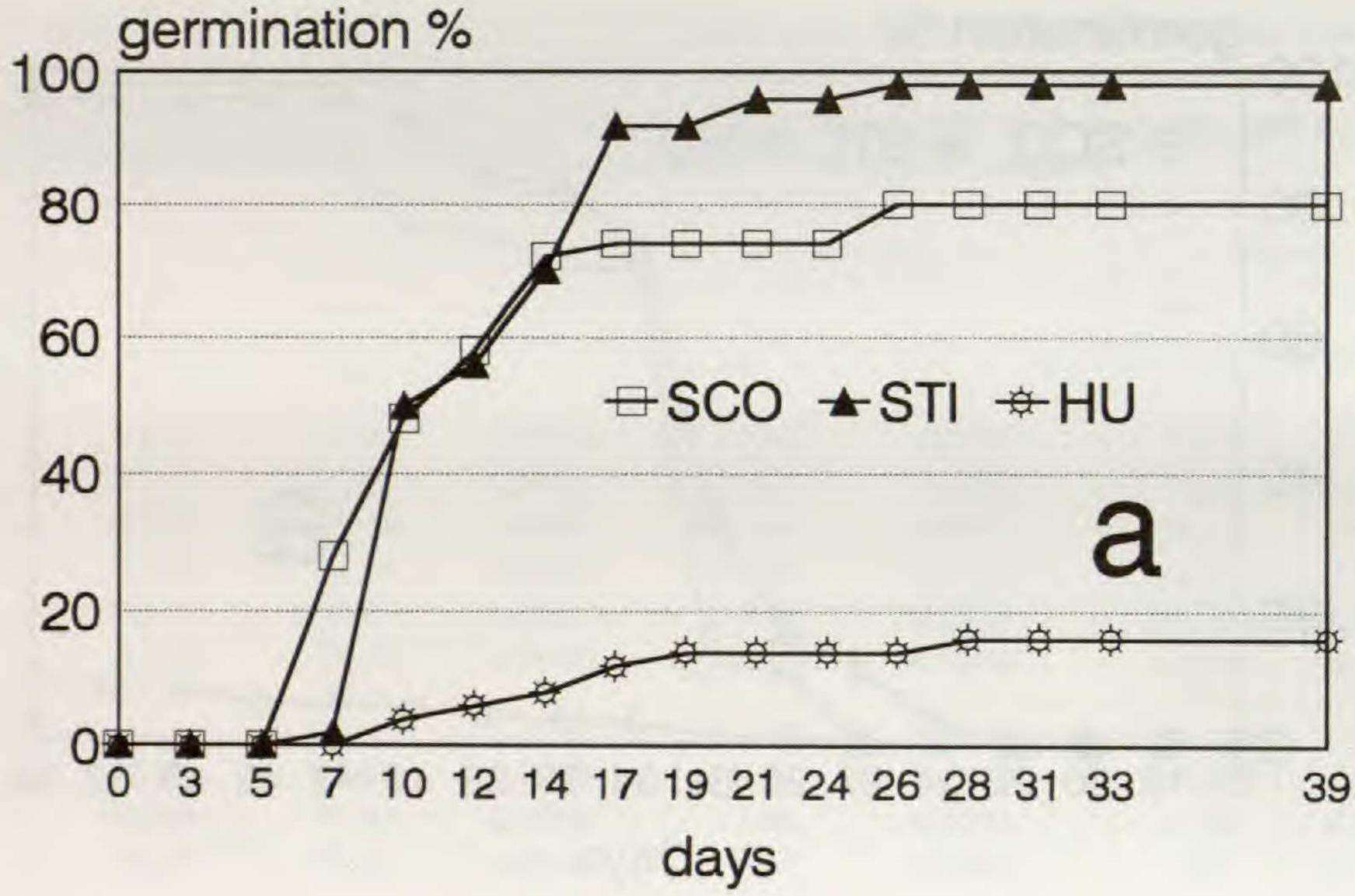


FIG. 2. Germination rates obtained in experiment 2. Temperature was 12°C until day 31, then 18°C. Light intensities: a) 40μEm^{-2}s^{-1}; b) 10μEm^{-2}s^{-1}.

Spores kept at 4°C germinated about 9 days earlier than the ones kept at −18°C. As in experiment 3, the germination rates of the latter treatment were lower, especially in population SCO, which decreased its germination from 80% to 10%.

It is noteworthy that when the temperature was changed, the germination rates increased abruptly in the samples kept at chilling temperatures, whereas



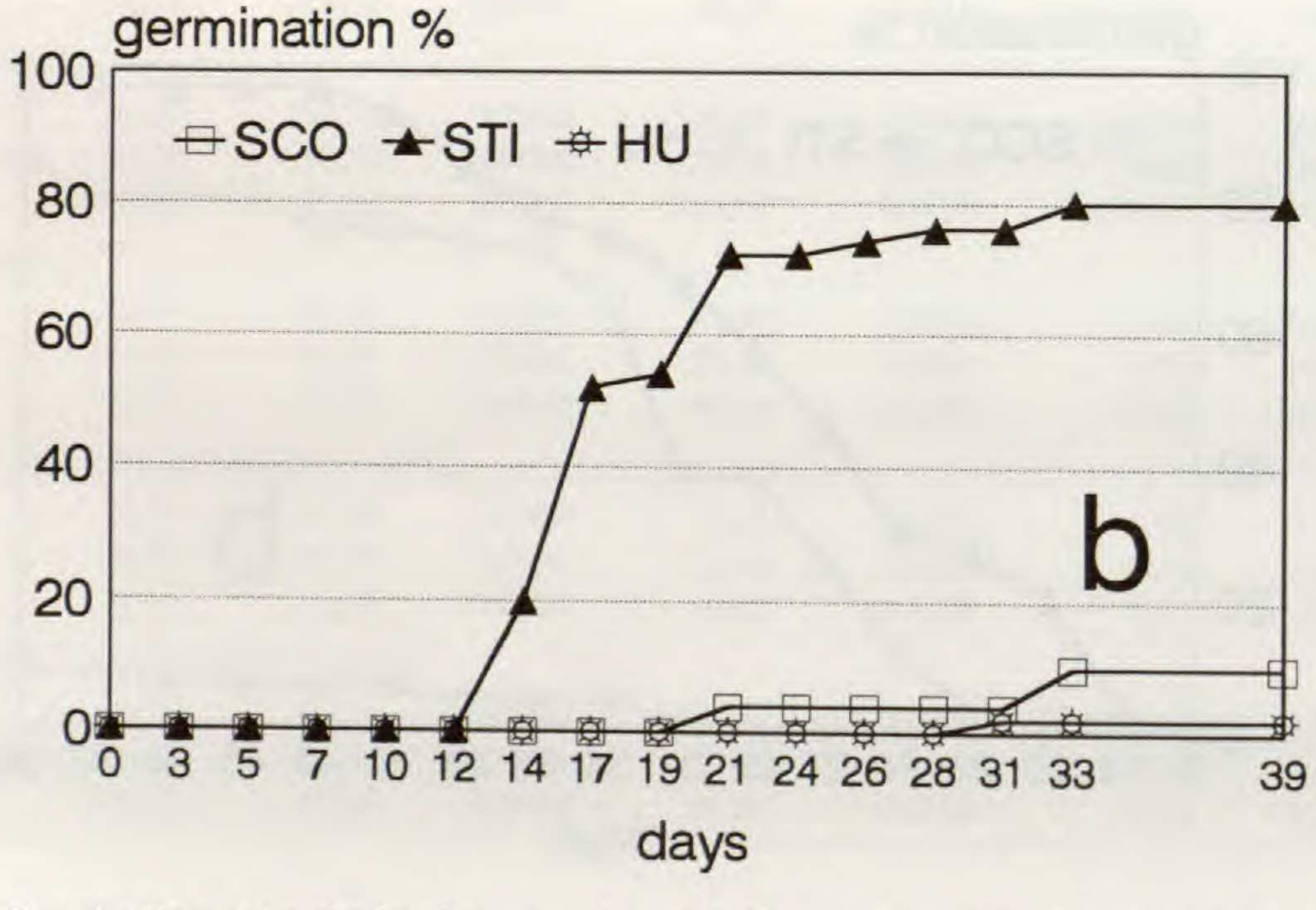


FIG. 3. Germination rates obtained in experiment 3. Temperature was 21°C and light intensity 30μEm^{-2}s^{-1}. a) Spores pretreated at 4°C. b) Spores pretreated at −18°C.

in the samples kept at freezing temperatures this increase showed up five days later. Comparing the results of experiments 3 and 4, it is apparent that similar overall germination percentages were reached at all combinations of temperature and light intensity. These germination rates were different in the spores pretreated at 4°C than in the ones kept at −18°C.

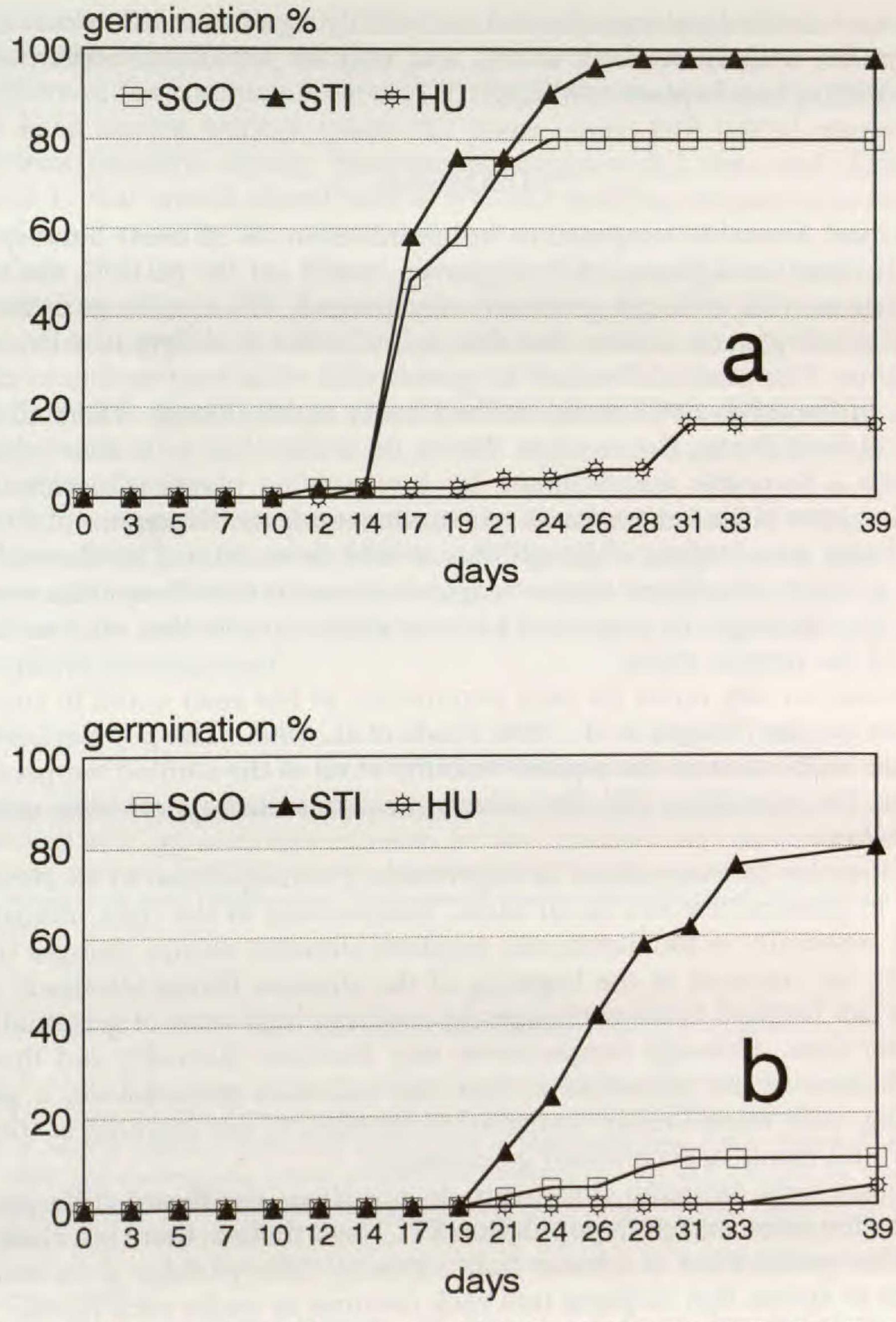


FIG. 4. Germination rates obtained in experiment 4. Light intensity was $10\mu Em^{-2}s^{-1}$. Temperature was 12°C until day 12, then 18°C. a) Spores kept at 4°C. b) Spores kept at −18°C.

As in experiment 3, statistical analysis showed significative differences among intercepts and among slopes of all three populations, although for STI it was only significant at the 95% level ($p<0.05$). When comparing results from experiments 3 and 4, no statistically significant differences appear among slopes in experiments with spores kept at −18°C, nor in experiments with

spores kept at 4°C. Intercepts showed statistically significant differences among experiments with spores kept at 4°C, and only in population STI in experiments with spores kept at −18°C.

Discussion

The most favorable temperature for germination, as in most homosporous ferns, is about 20°C (Dyer, 1979; Raghavan, 1989). At 10° or 15°C, the spores germinate as well, although germination is delayed. The significant differences among intercepts corroborate that this delay is due to differences in culture conditions. The small differences in germination rates may be due to chance events. In the wild, 20°C can be reached easily at the altitude where *C. crispa* grows, at least during the summer. Above the timberline, intensive insolation produces a favorable microclimate for low-growing plants (Geissler, 1982). However, even if the temperatures remain more or less cold, spores of *C. crispa* could begin germinating, although they would do so later. The absence of *C. crispa* at lower elevations, where temperatures around 20°C are common over a long period, might be explained by competition events that are outside the scope of the present study.

Germination rate varies for each population, as has been noted in some *Asplenium* species (Pangua et al., 1994; Prada et al., 1995). The populations PEÑ, STI, and MON showed the highest viability at all of the studied temperatures, whereas the population HU, for unknown reasons, had spores with very low germination.

The increase in temperature in experiment 2 corresponded to an abrupt increase in germination rate in all cases, independent of the light intensity. In nature, especially in Mediterranean montane climates, abrupt changes in temperature are common at the begining of the summer (Rivas-Martínez, 1987). Spores can respond to these changes by reaching high rates of germination in a shorter time. Although temperatures may fluctuate diurnally and there are other factors of the microhabitat that can influence germination, a general warming, with related mean temperature increase, at the begining of the summer, would result in accelerated germination.

Different light intensity treatments do not show significant differences in germination rates, except in population STI. Nevertheless, there is a clear trend for earlier germination at a lower light intensity. This perhaps gives some advantage to spores that disperse into rock crevices or under rock blocks.

The cold temperatures that spores endure during the winter can affect their viability in different ways. Spores kept at 4°C, a temperature probably common in hollows between rock blocks with snow cover that protects them from freezing, show germination rates similar to spores kept at room temperature (20°C). Spores kept at −18°C (experiments 3, 4), a temperature easily reached in these habitats where there is no snow cover protection during winter, delay their germination and lose viability at a higher or lower percentage (depending on the population) although some germination still occurs. Of course, in nature at high elevations, freezing temperatures may be acting over a longer period

than those in our experiments. This would presumably mean that spores in exposed sites would lose viability to a greater or lesser degree. But in rock boulder areas, the common habitat of *C. crispa,* there are a lot of safe sites that would keep spores warmer under the snow cover. Hill (1971) observed in *Thelypteris palustris* Schott, *Woodwardia virginica* (L.) Sm., and *Adiantum pedatum* L. that spores stored both at 6°C and freezing temperatures retained most of their viability. He concluded that it seemed that spores are able to retain their viability during winter, although evidence suggested that the usual overwintering stage is the gametophyte. We have not observed gametophytes in the wild in any of the studied populations, either during the spring or summer; this agrees with the observations of Peck et al. (1990) for *Cryptogramma stelleri* (S.G. Gmel.) Prantl. On the other hand, Dyer and Lyndsay (1992, 1996) detected *C. crispa* spores in British soil spore banks that retain their germination capacity.

Our results suggest that spores released by *C. crispa* at the end of the summer may delay their germination until the following spring. Spores that fall in cracks or other protected sites with a higher winter temperature (Young, 1985) and lower light intensity probably would germinate more quickly with faster gametophyte development.

Acknowledgments

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